4. Kisher CW: 1973, Collagen and dermal patterns in the hypertrophic scar. Anat Rec 179:137–146.
5. Knapp TR, Daniels JR, Kaplan EN: 1977, Pathologic scar formation. Am J Pathol 86:47–63.
6. Linares HA: 1988, Hypertrophic healing: controversies and etiopathogenic review. *In:* Burns in children. Practical burn management, ed. Carvajal JF, Parks DH, pp. 305–323. Year Book Medical Publishers, Inc., Chicago, IL.
7. Marder MZ, Cummings DW, Zegarelli DJ, et al.: 2000, Squamous cell carcinoma in an American bison (*Bison bison bison*). Vet Pathol 37:343–346.
8. Marjolin, J-N: 1828, Ulcère. *In:* Dictionnaire de Médecine 21: 31–50 (cited in reference 16).
9. Nakanishi H, Tomita Y, Yoshikawa H, et al.: 1999, Frequent p53 gene mutations in soft tissue sarcomas arising in burn scar. Jpn J Cancer Res. 90:276–279.
10. Novick M, Gard DA, Hardy SB, Spira M: 1977, Burn scar carcinoma: a review and analysis of 46 cases. J Trauma 17:809–817.
11. Ozek C, Celik N, Bilkay U, et al.: 2001, Marjolin's ulcer of the scalp: report of 5 cases and review of the literature. J Burn Care Rehabil 22:65–69.
12. Schumacher J, Watkins JP, Wilson SR, Foreman ME: 1986, Burn-induced neoplasia in two horses. Equine Vet J 18:410–412.
13. Schwartzkopf-Genswein KS, Stookey JM: 1997, The use of infrared thermography to assess inflammation associated with hot-iron and freeze branding in cattle. Can J Anim Sci 77:577–583.
14. Scott DW: 1988, Environmental diseases. *In:* Large animal dermatology, ed. Scott DW, pp. 67–68, 429–421. WB Saunders, Philadelphia, PA.
15. Spring PM, Myers JN, El-Naggar AK, Langstein HN: 2001, Malignant melanoma arising within a burn scar case report and review of the literature. Ann Otol Rhinol Laryngol 110:369–376.
16. Steffen C: 1984, Marjolin's ulcer. Report of two cases and evidence that Marjolin did not describe cancer arising in scars of burns. Am J Dermatopathol 6:187–193.
17. Steffen C: 1984, The man behind the eponym. Jean-Nicolas Marjolin. Am J Dermatopathol 6:163–165.
18. Yeruham I, Perl S, Nyska A: 1996, Skin tumors in cattle and sheep after freeze- or heat-branding. J Comp Pathol 114:101–106.

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Papular dermatitis induced in guinea pigs by the biting midge *Culicoides sonorensis* (Diptera: Ceratopogonidae)

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Abstract. Histological, ultrastructural, and virological examinations were performed on abdominal skin from guinea pigs after a blood meal by colony-bred biting midges, *Culicoides sonorensis*. Small, superficial, cutaneous, crateriform ulcers with necrosis of superficial dermis developed at feeding sites and healed within 24–48 hours. Animals developed nonpruritic erythematous papules 5 days after feeding that persisted until the study ended at 12 days after feeding. Papules corresponded histologically to foci of epidermal hyperplasia and superficial interstitial dermatitis with intraepidermal micropustules and scattered intraepidermal polykaryons. The principal ultrastructural changes were spongiosis in germinal epithelium and neutrophilic-histiocytic exocytosis. No viral agents or broken mouthparts were identified in lesions. The dermatitis may represent a host reaction to persisting insect salivary secretion and should be considered as an additional consequence of blood feeding in future studies involving biting midges.

Culicoides (Diptera: Ceratopogonidae) are small, dipterous flies (biting midges) that occur worldwide. More than 50 viruses have been isolated from *Culicoides* species, including the agents responsible for African horse sickness, bluetongue, epizootic hemorrhagic disease, and Akabane disease.[2,8,9] Female *Culicoides* incise the skin of mammalian and avian hosts using a sawing action by paired, toothed mandibular stylets. Unlike mosquitoes, which canulate the dermal microvasculature, biting midges are ''pool feeders'' that sever dermal vessels with cutting mouthparts to obtain a blood meal. Biting midges secrete saliva containing anticoagulants and other pharmacologically active compounds into the skin of hosts as they feed. The salivary gland components of *Culicoides sonorensis* have been partly characterized.[10,11] The morphology of cutting mouthparts of female *Culicoides* is illustrated in Fig. 1 and their presumed function during feeding has been described.[7,16]

Biting midges induce a well characterized, highly

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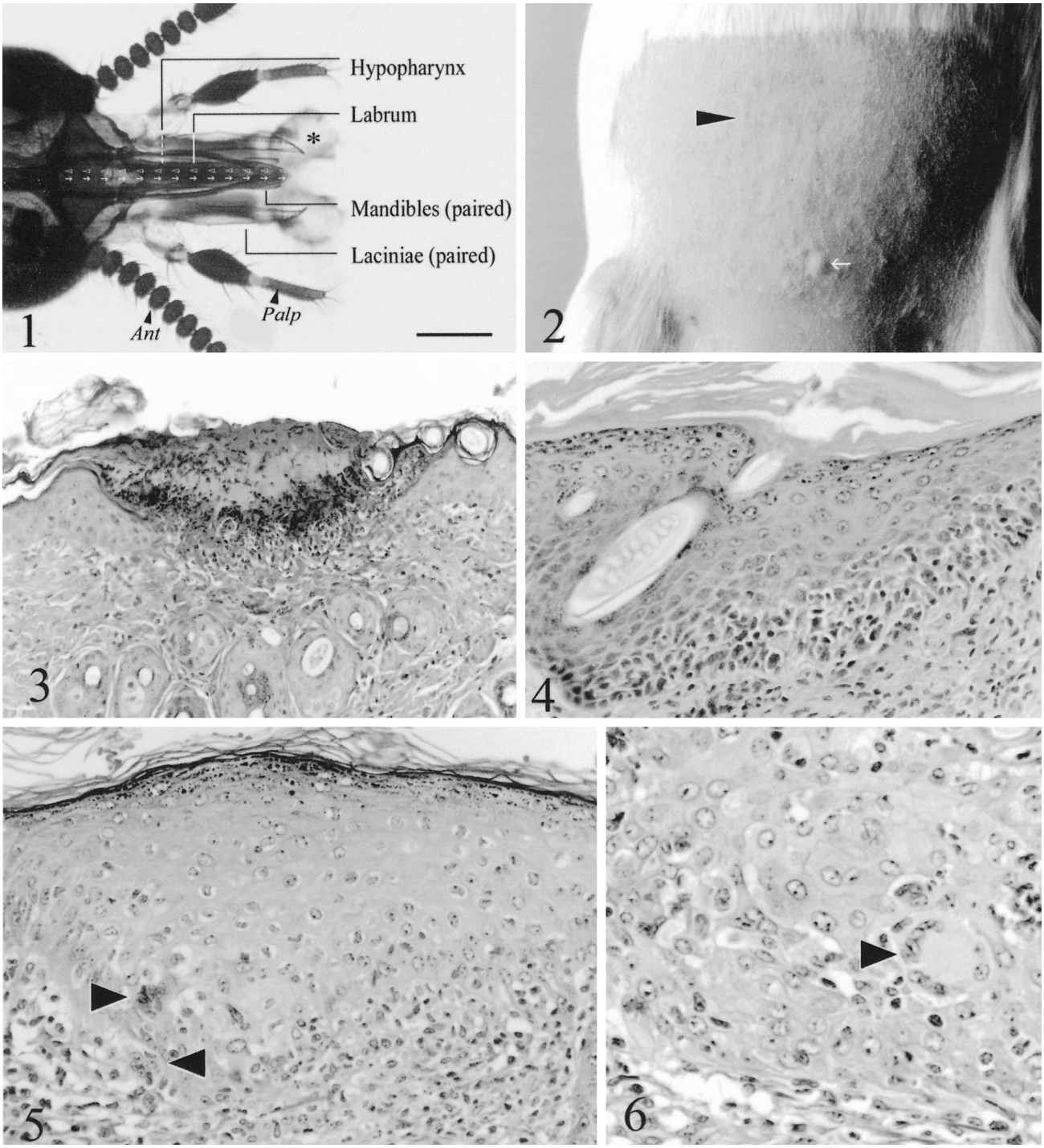

Figure 1. Dorsal view of head including syntrophium of *C. sonorensis*. There are 6 syntrophial stylets: the labrum (dorsal) and hypopharynx (ventral), paired mandibles, and paired maxillary laciniae. During feeding, skin is stretched by teeth at the tips of labrum and hypopharynx, and incised by a sawing action by the toothed mandibular stylets. Laciniae are pushed into the incision along with the mandibular stylets and anchor the wound during pool feeding. Symbols indicate the direction in which insect saliva is secreted (salivary duct-groove; arrows) and host blood is sucked (food channel; arrowheads) through compartments in the syntrophium. Maxillary palps (*Palp*) and antennae (*Ant*). The labium, a sensory structure that partly ensheaths the syntrophium, was removed to show stylets more clearly. Unstained mount. Bar: 50 μm.

Figure 2. Skin; animal No. 7; 12 days after feeding by *C. sonorensis*. There are multiple ≤1-mm cutaneous papules (solid arrowhead) within a circular area corresponding to the diameter of the feeding chamber. Umbilicus (white arrow).

Figure 3. Skin; animal No.1: immediately after blood meal by *C. sonorensis*. There is a focal crateriform cutaneous ulcer. Grossly, such lesions corresponded to red foci in skin. HE.

pruritic, delayed hypersensitivity dermatitis in mammalian species, primarily horses (''sweet itch'').[1,9,13,14] By contrast, little is known of the changes that occur in the skin of naive hosts after bites by midges. Petechial hemorrhages and papules develop shortly after people, sheep, or cattle are bitten.[1,9,10,13] A brief report on papular lesions in human skin appears to be the only description of the histological changes that occur in skin after exposure to biting midges.[15] In recent laboratory tests involving *C. sonorensis* blood feeding, a high proportion of naive guinea pigs develop a distinctive papular dermatopathy shortly after exposure. The study described here was undertaken to characterize the dermatopathy.

The experimental design is shown (Table 1). Between 177 and 369 colony raised, adult female *C. sonorensis* (AK colony) were allowed to blood feed once for 30 min through the fine mesh of a sealed feeding cylinder cage (inner diameter: 4.4 cm) resting on the shaved abdominal skin of each of 7 anesthetized 500–900 g HsdPoc:DH guinea pigs (animal Nos. 1–7).[a] Two anesthetized guinea pigs (Nos. 8 and 9) were exposed to empty feeding cages and served as unexposed controls. Guinea pigs were housed in a laboratory animal room and never previously exposed to biting midges. The parent animal colony was negative for mange mites (*Trixacarus caviae*) on the basis of monthly skin scrapings. The colony of *C. sonorensis* was established in 1973 from a field population and maintained for more than 25 years under large-scale rearing conditions.[5,6] After blood feeding, midges were killed by CO_2/immersion in ethanol, and examined using a dissecting microscope to determine the proportion that took a blood meal and the number with broken mouthparts.

Guinea pigs were examined clinically twice daily for signs of illness, including dermatitis. Feeding sites were palpated for abnormalities. Gross lesions were recorded photographically. Guinea pigs were anesthetized with ketamine/xylazine and euthanized by CO_2 at intervals up to 12 days after exposure to midges. One animal (No. 1) was killed and examined immediately after the cage containing insects was removed. Samples of skin and all major organs were collected into 10% neutral buffered formalin and processed for light microscopy. Sections of abdominal skin including feeding sites with and without lesions were stained with Periodic Acid Schiff (PAS), Gram, and Steiner stains. Abdominal skin including the feeding site was collected from 8 guinea pigs (Nos. 2–9) and examined for viral particles by negative stain electron microscopy. Skin from 2 animals with acute bite lesions (No. 1) or papules (No. 7), were fixed in 2.0% paraformaldehyde–2.5% glutaraldehyde in 0.1 M phosphate buffer and processed for transmission electron microscopy. Virus isolation was attempted from abdominal skin containing papules (where present) from 8 midge-exposed guinea pigs (Nos. 2–9) using 3 serial passages and a mammalian cell line (Madin-Darby bovine kidney (MDBK)) and 2 invertebrate cells lines (KC, derived from *Culicoides sonorensis* embryos; ATCC CRL 1660 clone C6/36 cells, derived from *Aedes albopictus* larvae).[b] After the third passage, homogenized cells were examined for viral particles by negative stain electron microscopy. Cells were freeze-thawed to increase the likelihood of viewing viral particles. Stock cells from the 3 cell lines were examined ultrastucturally and served as negative controls.

Guinea pigs remained alert, healthy, and free of pruritis during the study. Of 1,586 midges exposed to the skin of 7 guinea pigs, 1,206 (76%) took a blood meal. Four fed midges (0.3%) had broken mouthparts. Cutaneous lesions were restricted to the 4.4-cm diameter area that corresponded to the placement of feeding cages containing biting midges. Multiple (up to several hundred) ≤1 mm red foci in skin developed immediately after exposure to *C. sonorensis* (Table 1)

Table 1. Gross and microscopic lesions in guinea pigs exposed *C. sonorensis*.

Animal	Treatment	Insects exposed/ insects fed	Euthanasia DPE	Acute cutaneous ulcers	Papular dermatitis
1*	Insect exposed	233/221	Immediately	+†	−
2	Insect exposed	177/135	5	+	+
3	Insect exposed	177/157	5	+	−
4	Insect exposed	205/172	8	+	+
5	Insect exposed	195/152	8	+	+
6	Insect exposed	230/133	12	+	−
7*	Insect exposed	369/236	12	+	+
8	Feeding cage control	None	12	−	−
9	Feeding cage control	None	12	−	−

* Lesions sampled for transmission electron microscopy.
† +: Lesion present; −: Lesion absent.

←

Figure 4. Skin; animal No. 7; 12 days after feeding by *C. sonorensis*. The lesion corresponds to papules shown in Fig. 2. There is moderate epidermal hyperplasia and spongiosis, with a moderately intense lichenoid mononuclear inflammatory reaction in basal cell layer. HE.

Figure 5. Skin; animal No. 6; 12 days after feeding by *C. sonorensis*. Note 2 intraepidermal polykaryons (dark arrowheads). HE.

Figure 6. Skin; animal No. 6; 12 days after feeding by *C. sonorensis*. Higher magnification of an intraepidermal polykaryons (dark arrowhead). The abundant cytoplasm and marginated ring of nuclei suggests derivation from histiocytic-macrophage cells. HE.

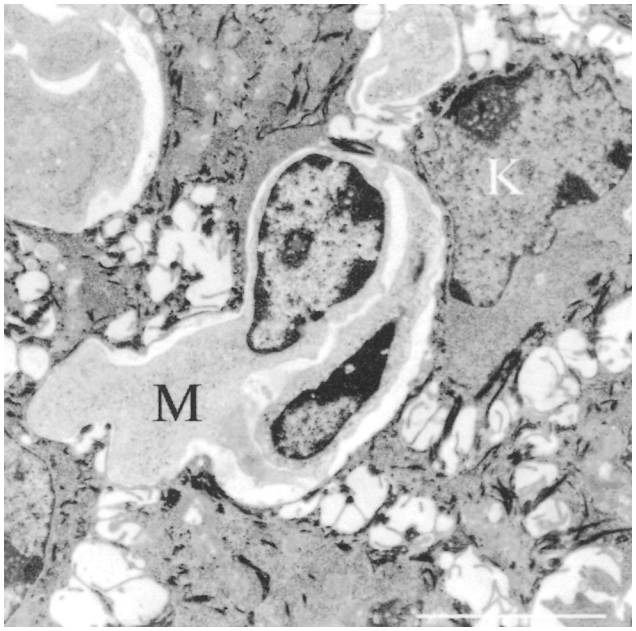

Figure 7. Skin; animal No. 7; electron micrograph of epidermal papule 12 days after exposure to *C. sonorensis*. Intense spongiosis separates keratinocytes (K). There is exocytosis by macrophages (M). No infectious agents, including viral particles, are visible. Bar: 5 μm.

and resolved completely within 1–2 days. No visible or palpable lesions were evident grossly between 2 and 4 days after exposure. Between 5 and 9 days after exposure, multiple (up to 50) ≤1–2 mm discreet, raised, pink, dry, nonpruritic lesions developed in abdominal skin in 4 of 6 guinea pigs (Fig. 2).

Histologically, shallow, crateriform, cutaneous ulcers (200 × 75 μm) developed immediately after feeding (Fig. 3). Some lesions were associated with exuded fibrin and necrosis of upper dermis and the superficial portions of hair follicles. Larger lesions (up to 1,000 μm diameter) were interpreted as confluent feeding sites. Intradermal hemorrhages were rarely identified in lesions.

Histological lesions developed in the skin of 4 of 6 guinea pigs on which *C. sonorensis* fed ≥5 or more days earlier. Typical lesions were 500–1,000 μm wide and 400 μm deep. They consisted of localized areas of moderate superficial perivascular to interstitial interface dermatitis, epidermal hyperplasia, spongiosis, neutrophilic-histiocytic exocytosis, and intraepithelial micropustules (Figs. 4, 5). Scattered polykaryons, some with marginated nuclei, were present in epidermis and there was loss of clear demarcation between epidermis and dermis (Figs. 5, 6). No infectious agents, viral-type inclusions, or insect mouthparts were detected in papules. Lesions were absent in noncutaneous tissues.

Ultrastructurally, bite lesions immediately after exposure to midges consisted of small ulcers with dermal necrosis to a depth of 100–200 μm. Dermal fibrocytes, leukocytes, and capillary endothelial cells were pyknotic and surrounded by fibrin. Epidermal papules contained a mixture of macrophages and neutrophils (Fig. 7). Keratinocytes in papules were individualized because of intense intercellular edema. The small number of pyknotic cells in epidermis consisted of leukocytes and keratinocytes in approximately equal proportion. Loss of demarcation between dermis and epidermis was due to dermal and epidermal edema, and migration of inflammatory cells across epidermal basal lamina. No foreign body material or infectious agents were identified ultrastructurally. Polykaryons were not represented in thin sections because of their relative paucity, and their fine structure was therefore uncharacterized.

Table 2. Detection of viral particles in cell lines after exposure of guinea pigs to *C. sonorensis*.

Animal/ preparation	Papules	Cell lines		
		BK	C6/36	KC
2*	−	−	+	−
3	+	−	−	−
4	+	−	+	−
5	+	−	+	−
6	−	−	−	−
7	+	−	+	−
8	−	−	+	−
9	−	−	+	−
C6/36 cell line (stock)	NA†	NA	+	NA

* Animal No. 1 was not examined.
† Not applicable.

Small, round viral particles (52–58 nm) were seen in negative stain preparations of homogenized C6/36 cells after exposure to skin from 4 of 6 guinea pigs exposed to *C. sonorensis,* as well as from both control animals (Table 2). The presence of identical viral particles in negative stain preparations of stock C6/36 cells indicated that the cell line and not skin was the source of this virus. No viral particles were identified in negative stain preparations prepared directly from homogenized skin of 6 guinea pigs exposed to *C. sonorensis* or from either control animal (Nos. 2–9).

Biting midges are pool feeders that lacerate skin to obtain a blood meal. Yet the nature of the lesions that developed immediately after feeding is difficult to explain by the cutting action of syntrophial stylets alone. Focal coagulative necrosis in dermis at feeding sites was suggestive of secretion by midges of a locally cytotoxic compound. The induction of focal necrosis at the meal site may aid biting midges to obtain a blood meal.

The cause of the papular dermatitis that developed 5 days after exposure to midges was not established

in the present study. None of the guinea pigs in the study was exposed previously to biting insects, and lesions were not characteristic of acute or delayed cutaneous hypersensitivity reactions.[3,4,14,17] Suspicion that papules were due to a viral infection endemic in the *Culicoides* colony, or a foreign body reaction to broken insect mouthparts, was unconfirmed. No viral agents were seen ultrastructurally in papules and the only virus identified after attempted isolation was a contaminant in 1 of 3 stock cell lines. The possibility that broken insect mouthparts elicited a foreign body reaction is excluded by their absence in lesions and by the small proportion of insects with broken mouthparts after a blood meal. Bacterial or protozoan agents were not found ultrastructurally or in special stains of affected skin, and lesions were not indicative of bacterial pyoderma. One explanation for the dermatitis is that pharmacologically active chemical components of *C. sonorensis* persisted in skin and elicited an inflammatory reaction. The possibility that the dermatitis was due to an infectious agent endemic in the insect colony and was undetected in the present study cannot be excluded.

A distinctive feature of the dermatitis was the presence of scattered, intraepidermal, multinucleated cells. These were a feature in bovine skin when cattle were exposed experimentally to *C. sonorensis* (O'Toole and Pérez de León, unpublished data). Unfortunately, polykaryons were sparse and not represented in blocks processed for electron microscopy, so their derivation from epithelium versus histiocytic cells was not established. Histiocytic derivation is likely, due to the relatively abundant cytoplasm and the presence of marginated nuclei. Intraepidermal, multinucleated, giant, histiocytic cells are a feature of various diseases that evoke cutaneous exocytosis, including drug eruptions.[17] Syncytial keratinocytes occur in viral infections such as monkeypox, some herpetic diseases involving human skin, and human and canine paramyxoviral infections.

In recent years there has been increased appreciation for the roles that arthropod salivary factors play in enhancing the likelihood of infection by various viral, bacterial, and protozoan agents.[12] The present study underscores the importance of establishing whether uninoculated insects induce lesions in naive hosts, even when long-established, well-characterized colonies are used.

Sources and manufacturers

a. Harlan Sprague Dawley, Indianapolis, IN.
b. American Type Culture Collection, Rockville, MD.

References

1. Akey DH, Luedke AJ, Osburn BJ: 1989, Development of hypersensitivity in cattle to the biting midge (Diptera: Ceratopogonidae). *In:* Physiological interactions between hematophagus arthropods and their vertebrate hosts, ed. Jones CJ, Williams RE, pp. 22–28. Miscellaneous Publication No. 71 of the Entomological Society of America, Washington, DC.
2. Boorman J: 1993, Biting midges (*Ceratopogonidae*). *In:* Medical insects and arachnids, ed. Lane RP, Crosskey RW, pp. 288–309. Chapman and Hall, London.
3. French FE: 1972, *Aedes aegypti*: histopathology of immediate skin reactions of hypersensitive guinea pigs resulting from bites. Exp Parasitol 32:175–180.
4. Gross TL, Ihrke PJ, Walder EJ: 1992, Feline mosquito-bite hypersensitivity. *In:* Veterinary dermatopathology, pp. 210–212. Mosby Year Book, St. Louis, MO.
5. Hunt GJ: 1994, A procedural manual for the large-scale rearing of the biting midge, *Culicoides variipennis* (Diptera: Ceratopogonidae). U.S. Department of Agriculture, Agricultural Research Service, ARS-121, pp. 1–68. National Technical Information Service, Springfield, VA.
6. Jones RH, Foster NM: 1978, Relevance of laboratory colonies of the vector in arbovirus research—*Culicoides variipennis* and bluetongue. Am J Trop Med Hyg 27:168–177.
7. McKeever S, Wright MD, Hagan DV: 1988, Mouthparts of females of four *Culicoides* species (Diptera: Ceratopogonidae). Ann Entomol Soc Am 81:332–341.
8. Mellor PS, Boorman J, Baylis M: 2000, Culicoides biting midges: their role as arbovirus vectors. Ann Rev Entomol 45: 307–340.
9. O'Donel Alexander J: 1984, Reactions to dipterous biting flies. *In:* Arthropods and human skin, pp. 123–124, 129–133. Springer-Verlag, Berlin.
10. Pérez de León AA, Ribeiro JM, Tabachnick WJ, Valenzuela JG: 1997, Identification of a salivary vasodilator in the primary North American vector of bluetongue viruses, *Culicoides variipennis*. Am J Trop Med Hyg 57:375–381.
11. Pérez de León AA, Valenzuala JG, Tabachnick WJ: 1998, Anticoagulant activity in salivary glands of the insect vector Culicoides variipennis sonorensis by an inhibitor of Factor Xa. Exp Parasitol 88:121–130.
12. Ribero JMC: 1995, Blood-feeding arthropods: live syringes or invertebrate pharmacologists? Infect Agents Dis 4:143–152.
13. Scott DW: 1988, Equine insect hypersensitivity. *In:* Large animal dermatology, pp. 302–306. WB Saunders Company, Philadelphia.
14. Stannard AA: 2000, Immunological diseases. Vet Dermatol 11: 163–178.
15. Steffen C: 1981, Clinical and histopathologic correlation of midge bites. Arch Dermatol 117:785–787.
16. Sutcliffe JF, Deepan PD: 1988, Anatomy and function of the mouthparts of the biting midge, *Culicoides sanguisuga* (Diptera: Ceratopogonidae). J Morphol 198:353–365.
17. Yager JA, Wilcock BP: 1994, Flea-bite hypersensitivity in the dog. *In:* Color atlas and text of surgical pathology of the dog and cat, pp. 24, 59–60. Mosby-Year Book Europe Limited, Spain.